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Evidence for the Lack of a Growth Principle in the Optic Cyst of Mexican Cave Fish.

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(Text-figures 1 & 2).

Introduction.

Because studies in another group of animals yielded evidence of some physiological influence on growth and sexual development which was evidently based on retinal elements, it became necessary to determine the presence or absence of such an influence in the Mexican cave fish (*Anoptichthys*) before proceeding with other experiments in which such an influence would have to be considered.

Specifically Browman (1940) reported that rats enucleated on the day of birth had a slower growth rate regardless of what light conditions they were reared under, and postulated that some "principle" was elaborated by the retina which was essential to normal growth and sexual development. On the other hand Detwiler and Copenhaver (1940) found that such treatment did not interfere with the growth of *Amblystoma* larvae.

Since in the studies concerned with the Mexican cave characins we are dealing with a series of fishes grading from fully eyed specimens from without the caves to blind cave forms with a remnant capsule which is still light sensitive and finally to a form which has lost this sensitivity, it is necessary in any experimental work concerned with the growth and development of these fishes to determine if the presence or absence of retinal elements is in any way involved. On a basis of general observation it had been noted that the eyed river fish attained the largest size of any and the light insensitive the least and reproductive activity in aquaria was successful in decreasing order in the same series. For a discussion of the differences and similarities between these graded forms in regard to morphology and behavior see Breder (1942, 1943, 1944 and 1945), Breder and Gresser (1941a and b), Breder and Rasquin (1943) and Gresser and Breder (1940).

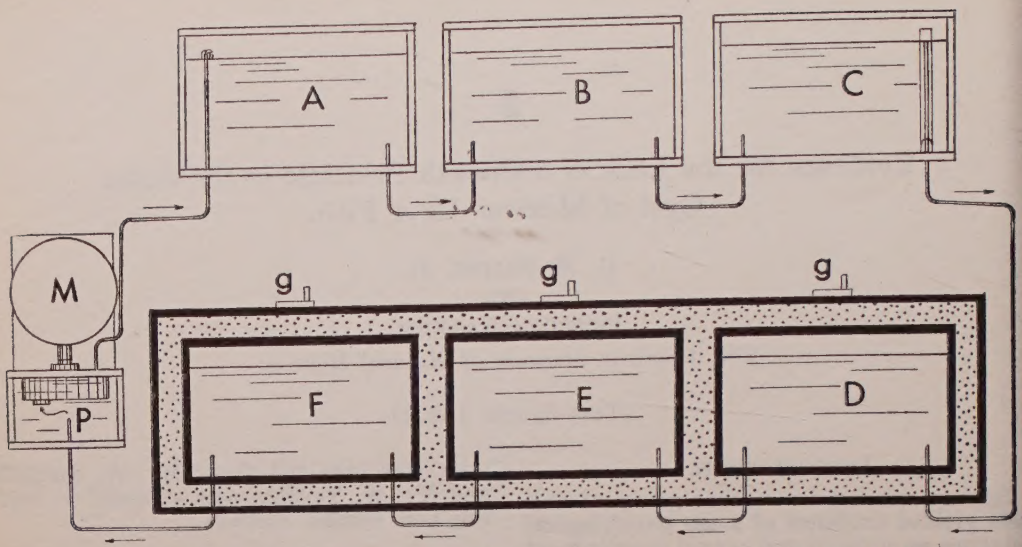
The experimental part of this work was carried on in the laboratories of the Department of Animal Behavior and assistance was obtained from Mr. W. Sutcliffe and Mr. W. Tavolga who attended to feeding and similar matters.

Materials and Methods.

Since the blind fish from La Cueva Chica, *Anoptichthys jordani* Hubbs and Innes, possess a blind but light sensitive cyst in the orbital cavity and since it may be removed with ease and impunity and results in, so far as is evident, only a loss of the light sensitivity, this form was used for the present studies. Another fact in determining this was that they are obtainable in quantity in a pure breed line. The fishes used actually represented the eleventh generation of tank reared fishes.

For any experiments involving the growth of fishes it is necessary to be particularly cautious that the ecological conditions established in one tank of standing water do not differ from another. As this is almost impossible to insure for any length of time, the problem was circumvented by connecting all the aquaria by glass tubing and slowly circulating the water through them. This guaranteed that the water in all would have the same temperature, chemical quantities and that the micro flora and fauna would be substantially similar. This arrangement was in fact essential because the experiment called for some of the aquaria being kept in the dark and others in the light which, without this provision, would have developed quite different ecological conditions.

In detail six aquaria measuring 2′ × 1′ × 1′ were connected in a closed circuit as is indicated in Text-fig. 1. The three upper aquaria, A, B and C, were exposed to light and an overflow from C connected with the three lower aquaria D, E and F which were in darkness. A small motor-driven hard rubber pump lifted the water from a receiving reservoir and delivered it to A at about 200 cc. per minute. A program switch allowed the pump to operate on alternate hours. The glass tubing was covered with black tape



TEXT-FIG. 1. Diagram of closed circulation for the maintenance of identical water conditions in aquaria in light and darkness. **M**, motor. **P**, pump. **g**, gate for feeding.

and bent through several right angles in order to prevent light entering the dark aquaria.

Since, for the duration of the experiment, it was necessary not to expose the fishes in the dark aquaria to light at any time, it was necessary to provide some means of introducing food. To this end an arrangement was provided in the cover over each aquarium in the dark to permit such feeding, which is indicated by g in Text-fig. 1. A three-quarter inch hole was bored through the cover as shown in Text-fig. 2. This was protected by two small boards, one on top and one under the cover, which were rigidly connected by a bolt as is shown in detail in Text-fig. 2B. The upper board was provided with a simple handle as indicated. Thus the two small boards would move in unison with the bolt as a pivot when the handle was appropriately operated. A stop in the form of a small screw prevented complete rotation. Text-fig. 2A shows the apparatus in one position and the curved arrow shows the amount and direction of travel. It will be noted that at all times one board or the other blocks the passage of light through the hole in the cover.

In actual operation the device was set in the position shown in Text-fig. 2A and the required amount of food placed in the hole in the cover. Then the device was rotated as shown by the arrow. When it had been swung through the complete arc the upper board covered the hole in the cover but the lower one did not, which permitted the contents of the hole to fall to the water below. Since granular dried food was used throughout this operated satisfactorily.

Twenty-five fish were placed in each of the six aquaria according to the following schedule.

Aquaria in light

A. Fish with ocular cyst removed.
B. Fish with optic nerve cut.
C. Control.

Aquaria in darkness

D. Fish with ocular cyst removed.
E. Fish with optic nerve cut.
F. Control.

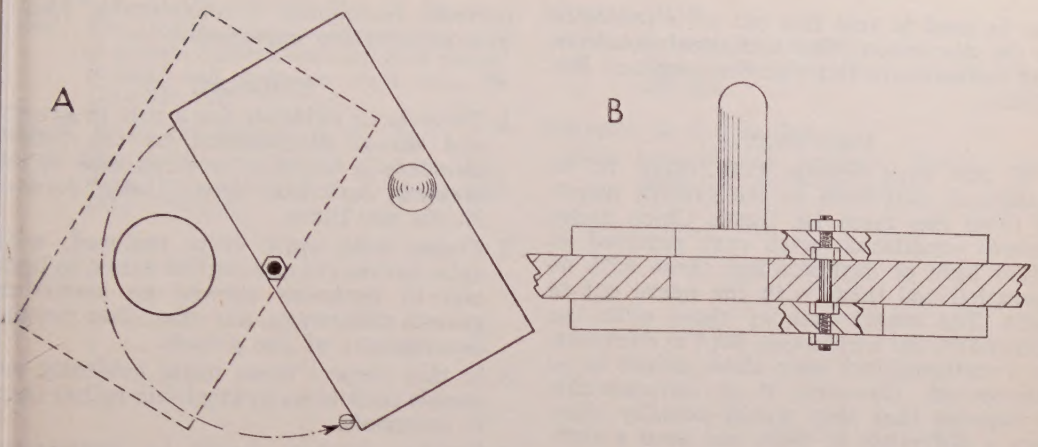
All the 150 fish were individually anesthetized with a 1 per cent. solution of urethane made up with the water from the aquarium. They were immersed in this until immobilized. They were then measured, weighed and operated upon. The aquaria, D, E and F, were not covered for two days to be certain that any possible delayed mortality could be checked.

The fish were measured again at an intermediate time and at the termination of the experiment. Except for the second measurement the fish kept in dark tanks were at no time exposed to light.

When the experiment was well along to completion an accident happened to the circulating system which resulted in the death of some fishes in the dark aquaria. This could have vitiated the entire operation were it not for the fact that the results obtained, because of their nature, are demonstratively independent of this unplanned incident. The only other losses were due to a kidney disorder, which has been mentioned by Nigrelli (1943).

EXPERIMENTAL RESULTS.

Since the experimental results were all obtained as numerical quantities, they may



TEXT-FIG. 2. Feeding gate. **A**, plan view. **B**, elevation in cutaway section.

est be presented as a table of comparative alues. These figures are given in Table I hich includes dates, duration, standard engths, mean weights, extent of mortality nd measures of significance of the various omparative values.

The fish were operated on as soon as they vere large enough to make it physically racticable, that is at about 20 mm. in tandard length. The experiment was terminated when the fish had nearly doubled hat length and increased in weight proportionally.

The aquaria in light, A, with cyst removed, B, with nerve cut and C, the control, n statistical analysis show that there has been no measurable difference in the growth f these fishes during this period of life vhere their growth is most active. Comparing these as samples they could have all been taken from a single population as in ach case d/σ_d is much less than 3. Actually the control tank showed slightly less growth in length than the other two while on the other hand it showed a greater growth in weight.

The tanks in darkness, D, with cyst removed, E, with nerve cut, and F, the control, were as previously noted damaged by an accident which killed all the fish in F and all but three in E. The tank D, which also was heavily involved in this trouble, nevertheless on statistical analysis shows no significant difference from those in light, yielding a value for d/σ_d very much less than 3.

Tank F in which only three fish remained cannot be compared in this manner because of the small numbers and if the χ^2 method is applied a P value of less than 0.01 is obtained which could, in a mathematical sense, be used to suppose that these fish in fact did have a different mean. However, other matters of a non-mathematical nature

TABLE I. CHANGES IN LENGTH AND WEIGHT UNDER VARIOUS CYST CONDITIONS.

MEAN GROWTH RATE

Numbers in parentheses indicate number of fishes.

AQUARIUM	MEAN MM. IN STANDARD LENGTH 8/12/45	12/5/45	2/13/46	INCREASE MM.	%	$\Sigma(d^2)$ DAY 0	DAY 189
A	20.4 (25)	34.5 (16)	38.2 (14)	17.6	84	53.000	118.375
B	20.5 (25)	34.3 (18)	38.2 (16)	18.3	88	68.260	297.000
C	20.5 (25)	34.7 (20)	39.8 (19)	17.4	80	26.240	144.526
D	20.0 (25)	34.4 (14)	36.4 (13)	16.3	82	58.090	133.077
E	20.8 (25)	30.0 (3)	32.7 (3)	11.8	56	43.600	9.145
F	21.0 (25)	.. ..	.. ..	..	..	24.960	..
	MEAN WEIGHT IN GRAMS			GRAMS	%		
A	0.43	1.25	1.54	1.11	258		
B	0.36	1.28	1.67	1.31	364		
C	0.25	1.27	1.77	1.52	604		
D	0.26	1.29	1.79	1.53	588		
E	0.35	0.93	1.32	0.97	278		
F	0.29	..	..	..	..		

can be used to rule this out as is indicated in the discussion. The statistical notations and methods are those of Simpson and Roe (1939).

Discussion.

It has been shown that there is no statistical difference to the growth means of blind fish from La Cueva Chica under several conditions; with cyst removed in either light or darkness and those with no operation and those with the nerve cut in light. The single case of those with the optic nerve cut which were kept in darkness, on a mathematical basis alone, might be so interpreted. However, it is unreasonable to suppose that they would actually show such a difference if there had been a sufficient number when it is recalled that those with the cyst completely removed show no such possibility and agree with three separate conditions in regard to the cyst of those kept in light. In fact the whole group shows remarkably uniform growth compared to the generality of aquarium reared fishes, which can only be credited to the circulating water arrangement.

Therefore it can be concluded that during the most actively growing period of these fishes, where they double their length in about six months, any possible effect of such retinal influence is either altogether absent or of such slight effect as to be impossible of detection by this method.

Consequently it seems likely, as might have been supposed on general considerations, that fishes behave more in a manner in accordance with amphibians than they do with mammals. Also it makes possible the disregarding of this factor in any ordinary problems of growth in these fishes.

After the experiment was terminated the fishes were retained for other purposes. Later, on examination in other connections, it was found that all types showed a perfectly normal gonadal development.

The purpose of these experiments was to determine if there was a growth or developmental function that could be ascribed to the remnant retinal elements in the optic cysts of the blind fish. Since, as has already been mentioned, the eyed river fish attain a somewhat larger size, a natural continuation would be to undertake experiments implanting or injecting retinal extracts into these blind fish in a manner analogous to the work of Browman and Browman (1944). If positive results were obtained one would then be in a position to indicate unequivocally that such an effect is present in fishes but the remnant left to the blind fish is too little to have its effects readily detected. The reverse experiment, that of blinding fish, is charged with so many practical difficulties that any retardation in growth, or the mere problem of keeping them in health for long periods, introduces a considerable hazard to a satisfactory experiment.

Summary.

1. There is no evidence for a role in growth and sexual development of the retinal elements in the blind optic capsule of the Mexican cave fish, *Anoptichthys jordani* Hubbs and Innes.
2. Fishes with optic cysts removed, with optic nerves cut and control fishes, in light and in darkness showed no statistical growth differences, nor other than normal development of the gonads.
3. In this respect these fishes evidently resemble conditions in amphibia rather than in mammals.
4. Further studies should be undertaken involving the injection or implantation of retinas from eyed fishes.
5. An arrangement for providing uniform ecological conditions in a series of aquaria in light and in darkness is described.

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